NKX019, an Off-the-Shelf CD19 CAR-NK Cell, Mediates Improved Anti-Tumor Activity and Persistence in Combination with CD20-Directed Therapeutic mAbs

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Introduction

NKX019 is an investigational CD19-targeting chimeric antigen receptor (CAR) natural killer (NK) cell therapy with engineered persistence for treating B cell malignancies (**Figure 1A**). NKX019 exhibits more rapid cytotoxic kinetics than CD19-directed CAR T cells, and lower production of cytokines associated with cytokine release syndrome (CRS) **[1]**. The safety and clinical activity of NKX019 as monotherapy are currently being evaluated in a Phase I clinical study [NCT05020678].

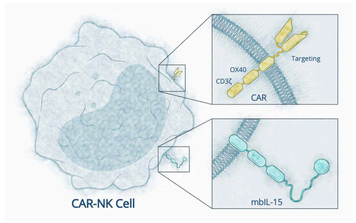
Recent studies **[2,3]** have shown that combining NK cell therapies with monoclonal antibodies (mAbs) may improve targeted NK cell activation and overcome some of the disadvantages associated with the stand-alone use of therapeutic mAbs, including antigen downregulation, exhaustion of complement, trogocytosis, upregulation of anti-apoptotic proteins, and host effector cell exhaustion **[4]**. CD20-targeted mAbs, such as rituximab (RTX) and obinituzumab (OBI), can mediate antibody-dependent cellular cytotoxicity (ADCC), a key effector mechanism of NK cells (**Figure1B**). This process is facilitated by the Fc receptor, CD16a, on human NK cells. A common polymorphism is found in CD16 that significantly affects affinity for Fc and can influence ADCC responses. Specifically, the polymorphism at position 158 of phenylalanine (158F) or valine (158V) results in CD16 with decreased or increased affinity for Fc, respectively. RTX also activates some levels of caspase-dependent direct cell death (DCD). OBI is engineered to induce improved DCD and ADCC mechanisms, irrespective of CD16a genotype **[5]**. Antigen escape is reported in 30–95% of relapses after CD19-directed CAR T cell therapy in B cell–acute lymphoblastic leukemia (B-ALL) **[6]**. Here, we describe the potential advantage of using NKX019 in combination with RTX or OBI to reduce relapse following monotherapy with either modality alone by targeting both CD19$^+$and CD20$^+$ malignant B cells.

Methods

- NK cells, isolated from healthy PBMCs, were expanded and engineered to express a CD19-targeted CAR and membrane-bound interleukin 15 (mbIL-15) to generate NKX019. NKX019 cells were cryopreserved and freshly thawed for experimental use.
- NKX019 mediated-cytotoxicity was assessed in both 4-Hour and extended assays in the presence or absence of anti-CD20 mAbs, RTX or OBI, using CD19$^+$ and CD20$^+$ expressing Chronic Lymphocytic Leukemia (CLL) primary cells and tumor B cell lines: Raji (lymphoblast-like), DOHH-2 (follicular lymphoma) and EHEB (B-Lymphoblastoid) cells.
- A non-glycosylated version of RTX (RTX mutant) with compromised ADCC function was used to evaluate ADCC-mediated vs ADCC-independent activity of NKX019.

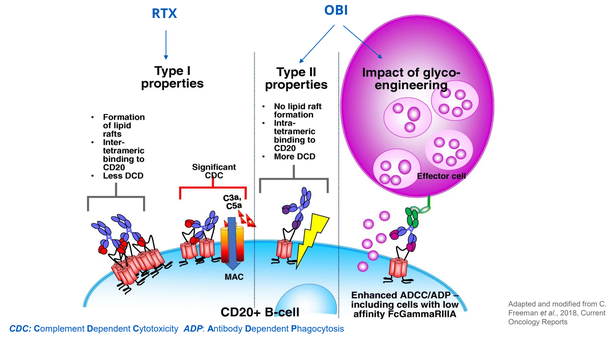
Figure 1

A. Diagram of NKX019, a donor derived CAR NK



Targeting receptor,
OX40 costimulatory domain,
CD3ζ signaling moiety,
Membrane bound IL-15 (mbIL-15)

B. Structure and binding of rituximab and obinituzumab



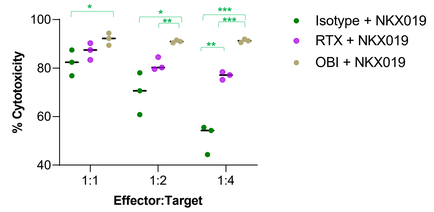
Adapted and modified from C. Freeman *et al.*, 2018, Current Oncology Reports

CDC: **C**omplement **D**ependent **C**ytotoxicity ***ADP:*** **A**ntibody **D**ependent **P**hagocytosis

Results

Superior Cytolytic Activity of NKX019 in the presence of CD20-Targeted Monoclonals

A. 4-hour Cytotoxicity of NKX019 Towards CD19$^+$CD20$^+$ Raji Cells



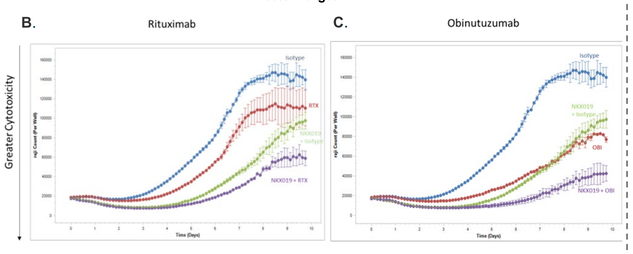


Figure2 NKX019 Cytolytic activity was measured using the Burkitt's lymphoma derived cell line Raji-NucleoRed (NR) cell line. **(A.)** Raji-NR cells were incubated with NKX019 cells in the presence of 1μg/mL of anti-CD20 antibodies or corresponding irrelevant isotype control, for 4 h at the indicated E:T ratios (average of % cytotoxicity from 3 donors is shown). Data is presented as the normalized frequency of target cells remaining, where target cells + isotype control without effector cells is = 100%. **(B.-C.)** Raji-NR growth curves after 10 days of co-culture with 0.01μg/mL of either isotype control (blue curve) or anti-CD20 alone (RTX **(B.)** or OBI **(C.)**, red curve), with effector cells alone (NKX019 + Isotype, green curve) or with a combination of NKX019 + anti-CD20 (purple curve) N=3, Representative Donor is shown, E:T = 1:4. *p< 0.05, ** p< 0.01, *** p< 0.001, unpaired t-test.

Enhanced cytolytic persistence of NKX019 in the presence of CD20-Targeted monoclonals in tumor rechallenge experiments

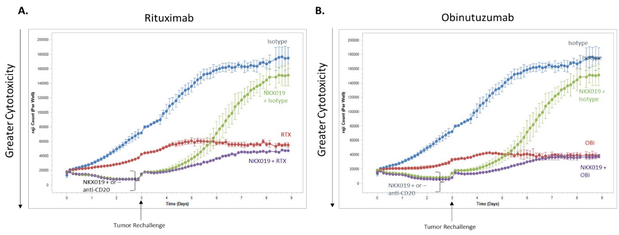


Figure3 NKX019 cytolytic activity and persistence were measured using Raji-NR cell line. Raji-NR growth curves after 9 Days of co-culture with 10 μg/mL of either isotype control (blue curve) or anti-CD20 agent alone, RTX **(A.)** or OBI **(B.)** (red curve), with effector cells alone (NKX019 + Isotype, green curve) or with a combination of NKX019 + anti-CD20 (purple curve). N =3, 1 representative donor is shown. E:T = 1:1 at time 0. All conditions were re-challenged at day 3 with 10x10^3 of tumor cells.

NKX019 in combination with RTX demonstrated an enhanced cytotoxicity against EHEB cell line in a manner consistent with ADCC

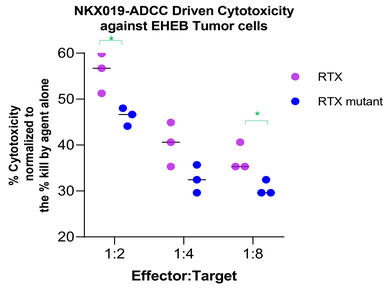


Figure4 EHEB cells were incubated with NKX019 cells in the presence of 1μg/mL of RTX, RTX mutant or corresponding irrelevant isotype control, for 4 hours at the indicated E:T ratios (average of % cytotoxicity from 3 donors is shown). Data is presented as the normalized frequency of target cells remaining, where target cells + anti-CD20 antibody is = 100%. *p < 0.05, unpaired t-test.

Obinutuzumab treatment increases NKX019 degranulation against Raji-NR, DOHH-2 and EHEB Cell Lines

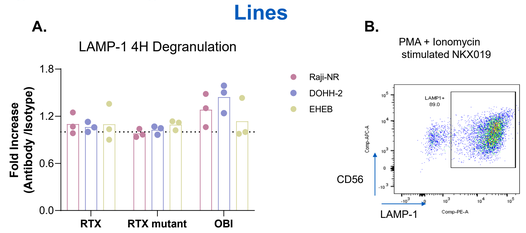


Figure5 **(A.)** NKX019 cells were stimulated with a 1:1 ratio of target cells in the presence of 1μg/mL of anti-CD20 antibodies or corresponding isotype control and stained for LAMP-1 after 4 hours. Quantification of LAMP-1 expression by NKX019 cells: increase in LAMP-1 positive cells in the antibody group was normalized to the Isotype-antibody group (fold increase: antibody/Isotype control) - Average of % cytotoxicity from 3 donors is shown) **(B.)** LAMP-1 staining on NKX019-cells stimulated with PMA + Ionomycin.

NKX019 exhibits enhanced ADCC against primary patient-derived leukemia cells, irrespective of CD16 polymorphism and baseline cytolytic activity

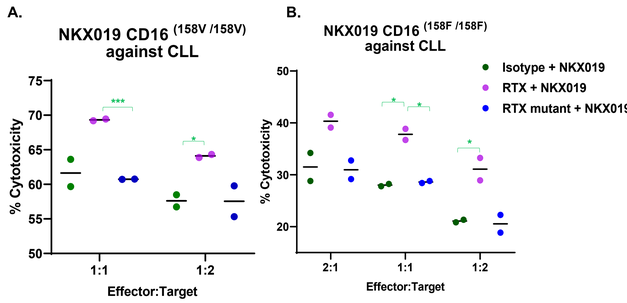


Figure6 Leukemic cells from 2 CLL patients were incubated with NKX019 from donors expressing a higher affinity 158V/158V **(A.)** or lower affinity 158F/158F **(B.)** CD16 genotype, in the presence of 1μg/mL of anti-CD20 antibodies or corresponding irrelevant isotype control, for 4 h at the indicated E:T ratios (average of % cytotoxicity from 2 CLL patients is shown). Data is presented as the normalized frequency of target cells remaining, where target cells + isotype control without effector cells is = 100%. *p < 0.05, ***p < 0.001, unpaired t-test.

Conclusion

- NKX019 in combination with RTX or OBI demonstrated increased activity and persistence against tumor B cell lines in a 4-Hour (4H) kill assay and in tumor rechallenge experiments.
- NKX019 in combination with RTX demonstrated an enhanced cytotoxicity against EHEB lymphoblastoid cell line in a manner consistent with ADCC.
- OBI demonstrated increased activity in comparison to RTX, as a single agent and in combination with NKX019 cells.
- NKX019 exhibit enhanced ADCC-mediated cytotoxicity in combination with RTX against CLL patient-derived leukemia cells, irrespective of CD16 polymorphism.

This study demonstrates increased activity and persistence of NKX019 when used in combination with approved CD20-targeted mAbs, RTX and OBI, against B cell malignancies. As part of the NKX019 clinical development program, clinical study of NKX019 in combination with RTX is planned.

References

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